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(57) Abstract

An isolated cell or cell line, wherein the cell is β_2-microglobulin deficient, neomycin-resistant and HAT-sensitive is provided. The cell FO-1 #12 is an example of a cell having these characteristics. A cell hybrid formed by the fusion of an FO-1 #12 cell or other cell described herein and a mammalian cell is provided. The patient-derived cell can be a tumor cell or other cell, such as a white blood cell. The patient-derived tumor cell can be a melanoma cell, a prostatic carcinoma cell, a colon carcinoma cell, a lung carcinoma cell, a breast carcinoma cell, a pancreatic carcinoma cell, or others. A method of treating AIDS in a patient, comprising administering to the patient a cell hybrid provided herein, wherein the patient-derived white blood cell is derived from the patient being treated, is provided. A method of treating solid tumor in a patient, comprising administering to the patient a cell hybrid as provided herein, wherein the patient-derived tumor cell is derived from the patient being treated, is provided.

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SEMI-ALLOGENEIC CELL HYBRIDS AS PREVENTIVE AND THERAPEUTIC VACCINES FOR CANCER AND AIDS

BACKGROUND OF THE INVENTION

Field of the Invention.

The invention relates to immunotherapy. More specifically, the invention relates to the use of a patient-derived cell/non-patient cell hybrid as a preventive as well as therapeutic vaccine. Most specifically, the invention relates to a novel cell line, FO-1 #12, for use in generating cell hybrids for use in preventive and therapeutic vaccines for cancer, autoimmune diseases, and acquired immune deficiency syndrome (AIDS). The invention also relates to the use of cell hybrids to enhance the proliferation and activation of cytotoxic T lymphocytes (CTL) against disease-specific cells.

Background Art.

For years cancer has evaded immunotherapeutic treatment. The last few years have registered a dramatic expansion in the understanding of tumor immunology, thanks to a series of major discoveries ranging from the identification and molecular characterization of tumor rejection antigens (1-4), to the discovery of co-stimulating molecules which are expressed on the surface of antigen-presenting cells and are crucial to the effectiveness of the immune activation associated with presentation of antigenic peptides by major histocompatibility complex (MHC) class I molecules (5,6). Taken together, the results of these studies imply that antigenicity (i.e., the ability to express a tumor antigen) and immunogenicity (i.e., the ability to induce an effective immune response) are not synonyms; in fact,

an increasing body of experimental evidence indicates that tumor antigens are present even in poorly or non-immunogenic tumors. A clearer understanding of these difficulties has led to efforts aimed at greatly amplifying the immunogenicity of tumor cells, by engineering them to express specific molecules (5-7). However, these experimental approaches are technically complicated and, therefore, of limited use for treating human cancer.

Jami and Ritz (8) first described the immunization of inbred mice with somatic cell hybrids derived from the fusion of syngeneic tumor cells with allogeneic cells. Specifically, they showed that inbred 129/Sv mice were resistant to the inoculation of a tumorigenic challenge with syngeneic teratocarcinoma cells following pre-immunization with (teratocarcinoma X C3H L-cell) semi-allogeneic somatic cell hybrids. Similar observations were reported by Parkman (9) with tumor cell hybrids obtained by the fusion of EL-4 lymphoma cells ($H\text{-}2^b$) with C3H fibroblasts ($H\text{-}2^k$). These hybrids specifically immunized C57BL/6 ($H\text{-}2^b$) mice against a lethal challenge with EL-4 lymphoma cells. Several subsequent studies also described this immunotherapeutic approach (10-13).

However, despite the fact that semi-allogeneic cell hybrids were used to immunize experimental animals as long ago as 1973 (8), there have been no attempts to use semi-allogeneic hybrids for treating human cancer or AIDS. Furthermore, no previously described semi-allogeneic tumor cell hybrid exists that can be used to treat human cancer or AIDS.

This invention provides a novel type of therapeutic and preventive vaccine based on patient-specific, irradiated semi-allogeneic cell hybrids for use in the treatment or prevention of human cancers and AIDS. The use of the present semi-allogeneic cell hybrids is technically straight-forward, and entails modest expense compared to most cancer or AIDS treatment regimens.

SUMMARY OF THE INVENTION

An isolated cell having the characteristics of the cell line designated FO-1 #12 is provided. An isolated cell or cell line, wherein the cell is deficient in β_2 microglobulin, resistant to a selectable dominant marker and deficient in a selectable recessive marker is provided. The cell FO-1 #12 is characterized as being β_2 microglobulin deficient, neomycin-resistant and HAT-sensitive.

A cell hybrid formed by the fusion of an FO-1 #12 cell or other cell described herein and a mammalian cell is provided. The mammalian cell can be a human patient-derived cell. The patient-derived cell can be a tumor cell or other cell, such as a white blood cell. The patient-derived tumor cell can be a melanoma cell, a prostatic carcinoma cell, a colon carcinoma cell, a lung carcinoma cell, a breast carcinoma cell, a pancreatic carcinoma cell, etc.

An FO-1 #12-like cell of the invention is provided, wherein the cell expresses a heterologous antigen. The heterologous antigen can be a tumor antigen or an infectious agent-specific antigen (e.g., HIV-specific or human papilloma virus (HPV)-specific).

A method of making a cell hybrid is provided. The method includes the steps of a) contacting a cell deficient in β_2 microglobulin, having a selectable dominant marker and having a selectable recessive marker with a patient-derived tumor cell or other cell, under conditions in which cell hybrids are formed; and b) selecting cell hybrids by determining the presence of the dominant marker and the presence of the recessive marker, whereby the presence of both the dominant and recessive markers is correlated with the presence of a cell hybrid. This method can further comprise the step of identifying cells that express HLA class I surface antigens.

A method of treating a solid tumor in a patient, comprising administering to the patient a lethally irradiated cell hybrid, wherein the patient-derived tumor cell is derived from the patient being treated, is provided. Also, a method is provided for treating AIDS in a patient, comprising administering to the patient a lethally irradiated cell hybrid, wherein the patient-derived cell is white blood cell derived from the patient being treated.

A method of enhancing the proliferation and activation of a patient's cytotoxic T lymphocytes specific for tumor-associated, HIV/AIDS-associated or autoimmune disease-associated antigen targets is provided. The method comprises contacting a population of lymphocytes from the patient with the cell hybrid of the invention for an amount of time sufficient to increase the numbers and cytotoxic activity of CTL in the population.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the survival curve of FO-1-neo cells exposed to increasing doses of γ-rays.

Figure 2 shows the surface expression of HLA class I antigens on parental cells (FO-1 #12 and 501) and on tumor cell hybrids (FO-1 #12 X 501) obtained from their fusion. Single-cell suspensions from each culture (indicated at the side of the figure) were subjected to indirect immunofluorescent staining and reacted with second antibody alone (blank), monoclonal antibody (mAb) W6-32 (anti-HLA-A,B,C + β_2 microglobulin), and mAb PA2.1 (anti-HLA-A2). Fluorescence intensity was determined by flow cytometry on a Becton-Dickinson cell analyzer. Note that FO-1 clone 12 (FO-1) cells do not express HLA class I antigens because they lack β_2 microglobulin expression; in contrast, tumor cell hybrids (FO-1 #12 X 501) grown in selective medium containing HAT and the neomycin analog G418 (600 µg/ml) express on the cell surface HLA class I antigens, including HLA-A2 (this antigen derives from 501 parental cells).

Figure 3 shows the survival curve of 2 melanoma hybrids (FO-1 #12 x WM2; FO-1 #12 x JJ) exposed to γ-rays.

DETAILED DESCRIPTION OF THE INVENTION

"Allo" Cell.

The invention provides cells capable of fusing with patient cells to form cell hybrids. The allo cell provided herein is the fusion partner of a patient ("self") cell in the present semiallogeneic cell hybrids.

The present allo cell is an isolated cell or cell line, wherein the cell is deficient in β_2 microglobulin, resistant to a selectable dominant marker and deficient in a selectable recessive marker. The cell or cell line is preferably human, or human-derived. An example of a cell having these characteristics is the cell line designated FO-1 #12.

The cell or cell line as described, wherein the dominant marker is drug or antibiotic resistance is provided. The antibiotic resistance can be to neomycin. There are numerous examples of expression of a selectable dominant marker associated with resistance to drug/antibiotic other than neomycin: hygromycin, methotrexate, α-amanitin, ouabain, etc.

The cell or cell line as described, wherein the recessive marker is sensitivity to aminopterin-containing medium (sensitivity to hypoxanthine + aminopterin + thymidine (HAT)-containing medium) is provided. There are other examples of recessive selectable markers, such as deficiency in thymidine kinase.

The cell FO-1 #12 is characterized as being β_2 microglobulin deficient, neomycin-resistant and HAT-sensitive. An example of a method for making such a cell is given in the Examples. A cell having the characteristics of the cell line designated FO-1 #12 and deposited on August 27, 1996 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852 under accession number ATCC CRL-12177 is also provided.

Disease-Specific Antigen-Expressing Allo Cells.

An allo cell of the invention is provided, wherein the cell expresses a heterologous antigen. The terms "antigen" and "antigen fragment" and "antigenic" as used herein, means a protein (peptide, polypeptide, etc.) capable of inducing an immunogenic T cell-mediated response. The antigen, when expressed, can be presented at least in part on the surface of a cell, bound to an HLA class I molecule. Thus, the heterologous antigen need not be thought of as a typical cell surface antigen. For example, prostate-specific antigen (PSA) is a cytoplasmic protein, but gives rise to cellular immunity, because fragments of it are presented on the cell surface of cancer cells, bound to HLA class I molecules.

The cell can express a heterologous antigen that is a tumor antigen. For example, the tumor antigen can be gp100/pmel17 (which is constitutively expressed by FO-1 #12), carcino-embryonic antigen (CEA), MUC-1, HER-2/neu, MAGE-1, MAGE-3, BAGE, GAGE, tyrosinase, MART-1, gp75, MUM, HPV-16, prostate-specific antigen (PSA), and other breast cancer-specific antigens, colon cancer-specific antigens, lung cancer-specific antigens, pancreatic cancer-specific antigens, prostate cancer-specific antigens, HPV-specific antigens (23), or other antigens.

The cell can express a virus-specific antigen. For example the cell can express an HIV-specific antigen. The HIV-specific antigen can, for example, be gag or an antigenic fragment thereof, pol or an antigenic fragment thereof, env or an antigenic fragment thereof or nef or an antigenic fragment of it (24, 25).

A cell expressing a polypeptide fragment of the heterologous antigen is also provided. This cell can be the allo cell that will be fused to the patient cell. The term "fragment" as used herein regarding antigens, means a molecule of at least 5 contiguous amino acids that has an antigenic function as described herein. As used herein to describe an amino acid sequence (protein, polypeptide, peptide, etc.), "specific" means that the amino acid sequence is not found identically in any other source. The determination of specificity is made routine, because of the availability of computerized amino acid sequence databases, wherein an amino acid sequence of almost any length can be quickly and reliably checked for the existence of identical sequences. If an identical sequence is not found, the protein is "specific" for the recited source.

An antigenic fragment can be selected by applying the routine technique of epitope mapping to the larger antigen to determine the regions of the proteins that contain epitopes that are capable of eliciting an immune response in an animal. Once the epitope is selected, an antigenic polypeptide containing the epitope can be synthesized directly, or produced recombinantly by cloning nucleic acids encoding the polypeptide in an expression system, according to the standard methods. Alternatively, an antigenic fragment of the antigen can be isolated from the whole antigen or a larger fragment by chemical or mechanical disruption. Fragments can also be randomly chosen from a known antigen sequence and synthesized. The purified fragments thus obtained can be tested to determine their antigenicity and specificity by routine methods or by the TIL education method described herein.

The heterologous antigenic polypeptides to be expressed in the present cells can be tested to determine their immunogenicity and specificity. Briefly, B lymphocytes and T cells are isolated from a patient who has an immune response to the present vaccine. The peptides expressed by the vaccine are stripped off of the vaccine cells and loaded onto B cells. Patient T cells are then tested for their ability to kill the B cells loaded with the vaccine peptides. If the T cells kill the B cells, the peptide antigen(s) eliciting the response are purified and sequenced. By identifying the peptide, synthetic vaccines can be generated.

A nucleic acid encoding a particular antigen of interest, or a region of that nucleic acid, can be constructed, modified, or isolated. That nucleic acid can then be cloned into an appropriate vector, which can direct the expression of the antigen in the allo cell. The vector is contemplated to have the necessary functional elements that direct and regulate transcription of the inserted gene, or hybrid gene. These functional elements include, but are not limited to, a promoter, regions upstream or downstream of the promoter, such as enhancers that may regulate the transcriptional activity of the promoter, an origin of replication, appropriate restriction sites to facilitate cloning of inserts adjacent to the promoter, antibiotic resistance genes or other markers which can serve to select for cells containing the vector or the vector containing the insert, RNA splice junctions, a transcription termination region, or any other region which may serve to facilitate the expression of the inserted gene or hybrid gene. (See generally, Sambrook *et al.* (*Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory,

Cold Spring Harbor, New York, 1989)). The vector can be delivered to the cell for expressing the antigen-encoding nucleic acid using commercially available systems as further described below and in the literature.

Cell Hybrids.

A cell hybrid formed by the fusion between the allo cell (e.g., an FO-1 #12 cell or other cell described herein) and a mammalian cell is provided. The fusion can take place under any conditions suitable for such fusions. One set of conditions under which cell fusion take place is described in the Examples. It is recognized, however, that other conditions are known or can be derived that permit fusion, and this does not change the nature of the resulting hybrid.

The mammalian cell can be a human patient-derived cell. In one embodiment of the hybrid, the patient-derived cell can be a tumor cell. The patient-derived tumor cell can be a melanoma cell, a prostatic carcinoma cell, a colon carcinoma cell, a lung carcinoma cell, a breast carcinoma cell, a pancreatic carcinoma cell, prostatic carcinoma etc. In another embodiment, the patient-derived cell can be another cell, such as a white blood cell.

Also provided is a fused cell hybrid of the heterologous antigen-expressing cell (allo-antigen cell) of the invention and a mammalian cell. A cell hybrid, wherein the mammalian cell is a patient-derived human cell is provided. The patient-derived human cell can be a white blood cell, more conveniently a peripheral white blood cell.

In the fused cell hybrid of the invention, heterologous antigen-expressing cell can express a tumor antigen. Alternatively the heterologous antigen-expressing cell can express an HIV- or HPV-specific antigen.

Thus, the present semi-allogeneic vaccine is made of three components: 1) a "self" component represented by the patient-derived (-specific) HLA haplotype; 2) an "allo" component represented by any human cell line which has a different HLA haplotype; and 3) an "antigen" component which is disease-specific and may or may not be patient-derived. In one embodiment, the allo and antigen components are engineered into an appropriately modified human cell line (e.g., a cell having the characteristics of FO-1 #12) which is fused with the patient-derived self component in order to generate patient-tailored semi-allogeneic cell hybrids. It is both practical and convenient, but not necessary, to use peripheral white blood cells as the self component, since blood-drawing is a minimally invasive and rather innocuous procedure. An appropriate antigen component can be, for example HIV-derived gag protein product (peptide or polypeptide) for preventive, as well as therapeutic AIDS vaccines; carcino-embryonic antigen (CEA) for preventive as well as therapeutic vaccination against many forms of carcinoma (colon, breast, lung, pancreatic, etc.); gp 100 for preventive as well as therapeutic vaccination against melanoma; and prostate-specific antigen (PSA) for preventive as well as therapeutic vaccination against prostatic cancer. The genetic engineering involved in producing the allo/antigen cell of the hybrid is routine and can be accomplished using commercially available vectors and other reagents. The method of fusing the

allo/antigen cell and the self cell to form the hybrid is also routine and described herein.

The cell hybrid provided herein can be lethally irradiated for use as a preventive or therapeutic vaccine for cancer or AIDS. The irradiation step takes place shortly before administration of the hybrid to a patient as further described in the Examples. Thus, an irradiated semiallogeneic cell hybrid is provided.

A method for making a cell hybrid is provided. The method includes the steps of a) contacting a cell deficient in β_2 microglobulin, having a selectable dominant marker and having a selectable recessive marker with a patient-derived tumor cell or white blood cell under conditions in which cell hybrids are formed; and b) selecting cell hybrids by determining the presence of the dominant marker and the presence of the recessive marker, whereby the presence of both the dominant and recessive markers is correlated with the presence of a cell hybrid. This method can further comprise the step of identifying cells that express HLA class I surface antigens. An example of this method is described in detail in the Examples.

Semi-allogeneic cell hybrids as preventive and therapeutic vaccines for cancer and AIDS.

A method of treating a solid tumor or AIDS in a patient, comprising administering to the patient a cell hybrid of the present invention, wherein patient-derived tumor cell or white blood cell is derived from the patient being treated, is provided. By "treating" is meant an improvement in the patient's condition. The improvement can be in any of the parameters typically used by

clinicians to asses the condition of the patient. For example, reduction in or stabilization of tumor mass or in antigen level in serum are evidence of efficacious treatment of a solid tumor. In the case of AIDS, reduction in HIV titre or increase in $CD4^+$ counts in the peripheral blood are evidence of efficacious treatment of HIV infection or AIDS.

A method of treating or preventing a solid tumor in a patient, comprising administering to the patient a cell hybrid, wherein the patient-derived white blood cell is derived from the patient being treated and the fusion partner expresses a heterologous tumor antigen. The antigen expressed can be selected from the class of cancer-specific antigens, including, but not limited to those specifically named herein.

A method of treating or preventing AIDS in a patient, comprising administering to the patient a cell hybrid, wherein the patient-derived white blood cell is derived from the patient being treated and the fusion partner expresses a heterologous HIV-specific antigen. The antigen expressed can be selected from the class of HIV-specific antigens, including, but not limited to those specifically named herein.

The present invention provides preventive and therapeutic vaccines for cancer or AIDS, based on irradiated semi-allogeneic cell hybrids, generated by the fusion of patient-derived tumor or white blood cells, with the allo cell provided herein (26). Semi-allogeneic cell hybrids can be inactivated by irradiation and injected into the same patient to induce a specific anti-tumor or anti-HIV response, respectively. The present hybrids

eliminate the need to establish patient-derived tumor cell cultures, which notoriously constitute a major technical hurdle. Furthermore, FO-1-derived HLA class I antigens may enhance the anti-tumor or anti-HIV response by virtue of the allogeneic presentation of tumor or HIV antigens; and cell hybrid vaccines exposed to a single lethal dose of ionizing radiation can express HLA class I surface antigens for several days before dying.

The cancer or AIDS prevention or treatment method, wherein the cell hybrid is administered in conjunction with a cytokine is also provided. The cytokine can be interleukin-12 (IL-12), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-2 (IL-2), or a combination of these and other adjuvants.

Administration of the semi-allogeneic hybrid.

Cell hybrids derived from the fusion of FO 1-12 cells with patient-derived melanoma cells are selected by virtue of their HAT-resistant and neomycin-resistant phenotype as described below. At the time of vaccination, the hybrids are thawed and used to prepare the irradiated vaccine for injection as described below.

The vaccine consists of $5x\ 10^6$ (or more) irradiated tumor cell hybrids. Irradiated cells are resuspended in 0.1 ml physiological saline and injected intradermally (i.d.) into the surface of the shoulder or other cutaneous area as deemed appropriate by the physician.

Multiple vaccinations may be required to induce immunity. Follow-up vaccinations can be made until complete remission or stabilization is achieved.

Activation of CTL against patient-derived, disease-specific cells.

A method of enhancing the proliferation and activation of a patient's cytotoxic T lymphocytes specific for tumor-associated, HIV/AIDS-associated or autoimmune disease-associated antigen targets is provided. The method comprises contacting a population of lymphocytes from the patient with a cell hybrid of the present invention for an amount of time sufficient to increase the numbers and cytotoxic activity of CTL in the population. The amount of time can vary, but is expected to be in the range of from 2 to 10 days, more preferably from 3 to 7. An example of this method is provided in the Examples.

As used in the present context, the term "contact" includes close proximity as well as actual mechanical contact.

Because of the ability of the present hybrids to activate CTL, the hybrids can be used in a method of treating a solid tumor in a patient. The method can comprise the steps of: a) obtaining a population of lymphocytes from the patient; b) contacting the lymphocytes obtained from the patient with a cell hybrid of the invention for an amount of time sufficient to enhance the proliferation and activation of the patient's cytotoxic T lymphocytes; and c) returning the cells of step b) to the patient, whereby the tumor is treated.

The step of obtaining a population of lymphocytes from a patient is accomplished by any of the well known methods of obtaining peripheral blood-derived lymphocytes. A specific example of one such method is described in the Examples. The length of contacting time is essentially a

described above. The step of returning the activated lymphocyte population to the patient can be by known methods.

Having provided a method of enhancing the proliferation and activation of CTL, the invention also provides a composition comprising a population of cytotoxic T lymphocytes produced by this method. This composition is a valuable reagent in the screening and identification of tumor-associated, HIV/AIDS-associated, or autoimmune disease-associated antigens or antigenic peptides.

Thus, a method for educating patient-derived lymphocytes to enhance the activation of cytotoxic T lymphocytes specific for patient-derived tumor-associated, HIV/AIDS-associated, or autoimmune disease-associated antigens is provided. The patient-derived lymphocytes are educated by exposing them to irradiated semi-allogeneic cell hybrids derived from the fusion of patient-derived cells with a cell having a selectable dominant marker and having a selectable recessive marker (for example, the cell line FO-1 #12). The patient-derived cell used to form the semi-allogeneic cell hybrids can be a tumor cell or peripheral blood mononuclear cell (PBMC). The patient-derived tumor cell can be a melanoma cell, a prostatic carcinoma cell, a colon carcinoma cell, a lung carcinoma cell, a breast carcinoma cell, a pancreatic carcinoma cell, a prostatic carcinoma cell etc. The patient-derived PBMC can be from patients with cancer, HIV-infection or AIDS, autoimmune disease, etc. An example of this method is described in the Examples.

Screening for tumor-, HIV- or other disease-associated antigens

A method of screening for tumor-associated, HIV/AIDS-associated or autoimmune disease-associated antigens or antigenic peptides is provided. Briefly, CTL can be obtained from the peripheral blood of any patient who responds clinically to any form of the present vaccine; in parallel, B lymphocytes from the same blood sample can be obtained and immortalized by infecting them with Epstein-Barr virus (EBV). Patient-derived CTLs can be activated by exposing them to irradiated, semi-allogeneic cell hybrids (vaccine) and tested for *in vitro* lysis of the patient's own B lymphocytes after they have been mixed with antigenic peptides extracted from the vaccine itself. Biological evidence of antigenic-mediated lysis can be used as a crucial indicator to pursue the identification by routine physical-chemical means (e.g., mass spectrometry) of the sequence of the antigenic peptides eliciting the cytotoxic response.

Thus, a method for using educated cytotoxic T lymphocytes as cellular reagents in the identification of tumor-associated, HIV/AIDS-associated, and autoimmune disease-associated antigens or antigenic peptides is provided.

EXAMPLES

Construction of FO-1 #12 cells

FO-1 human cells are deficient in β_2 microglobulin production; therefore, they do not express HLA class I surface antigens (27). Expression of a transfected human β_2 microglobulin gene in FO-1 cells leads to restored expression of HLA class I antigens (28).

Derivation of hgprt$^-$ FO-1 cells:

FO-1 cells were mutagenized by exposing them to a single dose (3 Gy) of γ-radiation (6 Gy/min dose rate) and subsequently plated in complete medium containing the purine analog 6-thioguanine at a concentration of 5 µg/ml. The incorporation into the DNA allows the selection of cells that are deficient for hypoxanthine-guanine phosphoribosyl transferase (hgprt). Several hgprt-deficient (hgprt$^-$) FO-1 mutants were isolated and characterized for their sensitivity to hypoxanthine, aminopterin, and thymidine (HAT)-containing medium. A particular hgprt$^-$ FO-1 clone with a spontaneous reversion rate to a HAT-resistant (hgprt$^+$) phenotype of $<1x10^{-7}$ was selected. However, the actual reversion rate must be much lower, since after several months of experimentation, not even a single HAT-resistant FO-1 (hgprt$^+$) revertant was found.

Transfection of FO-1 hgprt$^-$ cells:

Exponentially growing FO-1 hgprt$^-$ cells were transfected, using the calcium phosphate precipitation technique (29), with a plasmid containing the neomycin-resistance gene (30). Neomycin-resistant clones were selected in Dulbecco's modified Eagle's medium (DMEM) with added 10% fetal bovine serum (FBS), 50 units/ml penicillin, 50 µg/ml streptomycin, and 40 µg/ml ciprofloxacin (complete medium), containing the neomycin analog geneticin (Gibco) at a concentration of 600 µg/ml. Neomycin-resistant clones became visible 3 weeks after transfection; and individual clones were expanded for further characterization.

Subsequently, a cell survival curve for FO-1-neo transfectants exposed to γ-rays was generated.

Specifically, single-cell suspensions in complete medium were irradiated with a single dose of γ-rays, ranging from 3 to 20 Gy, using a ^{137}Cs γ-radiator (J.L. Shepherd and Assoc.) delivering 6 Gy/min. Irradiated cells were plated in cell culture dishes with complete medium, and surviving clones were scored two weeks after irradiation by their ability to form colonies, which were counted following fixation and staining with Giemsa (Fig. 1). The result of these cell survival experiments indicated that a single dose of 25 Gy would be sufficient to inactivate ~$1x10^{12}$ cells. Therefore, this radiation dose was selected as a standard for inactivation of cells.

The coexistence in these engineered cells of a dominant marker (e.g., neomycin-resistance) and a recessive mutation (e.g., $hgprt^-$) are necessary and sufficient for the easy selection of semi-allogeneic cell hybrids (e.g., following PEG-mediated fusion of FO-1 transfectants with patient-derived tumor or white blood cells as described below) provided by the present invention.

Although only a single clone (FO-1 #12) was selected for use in the subsequent experimentation, given the teaching of the present specification, it is expected that other cells having the key characteristics of the exemplary FO-1 #12 cells are within the scope of routine repetition of the above described steps. For example, FO-1 #5, which has selectable dominant and recessive markers and is β_2 microglobulin deficient has also been made, and exhibits comparable biological properties.

Generation of tumor cell hybrids

Polyethylene glycol(PEG)-mediated cell fusion (31) between neomycin-resistant, hgprt$^-$ (HAT-sensitive) FO-1 #12 cells, and patient-derived cells, was conducted according to the procedure by Prado et al (32). When fusing FO-1 #12 cells with patient-derived cells in suspension (e.g., patient-derived white blood cells), the so-called stirring protocol is used (33).

Preliminary experiments of PEG-mediated cell fusion were carried out between FO-1 #12 and 501 human melanoma cells. Neomycin-resistant and HAT-resistant melanoma cell hybrids (FO-1 #12 x 501) were subjected to immunofluorescent staining using anti-HLA-A,B,C + β_2 microglobulin mAb W6-32 and anti-HLA-A2 mAb PA2.1, followed by affinity-isolated fluorescein-labeled goat anti-mouse immunoglobulin (Fig. 2).

The expression by FO-1 #12 x 501 cells of HLA-A2 surface antigen derived specifically from 501 parental cells confirmed that true hybrids had been obtained.

More recently, cell hybrids derived from the PEG-mediated fusion of FO-1 #12 cells with patient-derived tumor cells were generated and characterized. These patient-derived cells were obtained from tumor lesions removed as part of standard surgery and were in excess of the patients' needs. The resulting hybrids expressed HLA class I antigens.

Tumor cell hybrids from over eighty independent experiments of PEG-mediated cell fusion have been obtained, including: two human melanoma cell lines; one human prostatic carcinoma cell line; over forty patient-

derived primary melanoma cell suspensions; two patient-derived primary colon carcinoma cell suspensions; over twenty patient-derived lung carcinoma cell suspension; two white blood cell lines; and over ten HIV-infected patient-derived peripheral white blood cell suspensions.

Survival curves of tumor cell hybrids following exposure to γ-radiation were generated and the results of these studies indicate that tumor cell hybrids are as radiation-sensitive as parental FO-1 #12 cells; therefore, 25 Gy γ-ray (at 6 Gy/min) was adopted as a standard lethal dose to inactivate hybrids for vaccine purposes.

Semi-allogeneic cell hybrids tailored to and specific for each patient can be generated with cell suspensions from any solid tumor or from white blood cells; they can be propagated and irradiated before injecting them into each patient for the purpose of therapeutic as well as preventive vaccination. Moreover, irradiated tumor cell hybrid vaccines can be formulated with appropriate cytokines (IL-12, GM-CSF, IL-2, etc.) for enhanced efficacy.

Derivation of white blood cells (peripheral mononuclear lymphocytes) from peripheral blood:

Peripheral mononuclear lymphocytes (PML) are obtained from 20 ml of heparinized human blood. After diluting blood with Hank's balanced salt solution (HBSS) at a 1:1 ratio, the suspension is layered over the separation medium (Lymphocyte Separation Medium- LMS- Organon Teknika) and spun down at 400xg at room temperature for 15-30 min. Centrifugation sediments erythrocytes and polynuclear leukocytes and bands mononuclear cells which can be aspirated, transferred to a centrifuge tube and

diluted with an equal volume of HBSS. The mononuclear lymphocyte suspension is spun down for 10 min at room temperature at a speed sufficient to sediment the cells without damage (i.e., 160-260xg). Cells are washed again in HBSS, resuspended in appropriate diluent and counted before using them for fusion.

Derivation of tumor cell suspensions from surgically excised lesions:

The present protocol is a modification of the tumor disaggregation protocol by Yannelli et al (34). Tumors are retrieved immediately after excision from each patient, put in Hank's balanced salt solution (HBSS) on ice, and transported to the laboratory. Tumor specimens are then transferred under sterile conditions to a 100 mm culture dish containing HBSS. After separating fat and necrotic tissue away from tumor tissue (1-2 grams), the latter is minced into pieces as small as possible using scalpel blades.

Minced tumor tissue fragments are transferred to flasks containing 25-50 ml of an enzymatic solution made of RPMI medium without serum, containing type I collagenase (1.0 mg/ml, Worthington) and DNase I (0.1 mg/ml, Sigma). The flask containing tumor cells is incubated at room temperature for 16-18 hours on a magnetic stir plate. The digested tumor cell suspension is then filtered through a sterile Nitex 40 nylon filter (mesh size 95 µm) to exclude undigested tumor fragments. The cell suspension is transferred to 50 ml conical centrifuge tubes and spun at 250xg for 10 min at 4°C in a refrigerated centrifuge, washed once with HBSS, resuspended in an appropriate volume of HBSS and layered over Lymphocyte Separation Medium (LMS, Organon Teknika)

and spun down at 400xg at room temperature for 15-30 min. Centrifugation sediments erythrocytes and polynuclear leukocytes and bands mononuclear blood cells and tumor cells which can be aspirated, transferred to a centrifuge tube and diluted with an equal volume of HBSS. The cell suspension is spun down for 10 min at room temperature at a speed sufficient to sediment the cells without damage (i.e., 160-260xg). Cells are washed again in HBSS, resuspended in appropriate diluent and counted and checked for viability by trypan blue exclusion test. Separate aliquots of the single cell suspension are used for a) fusion with FO-1 #12 cells to derive tumor cell hybrids, b) growth of tumor-infiltrating lymphocytes, and c) freezing for later use as autologous targets in cytotoxicity assays (see below). During processing, all solutions include gentamicin (50 µg/ml).

Formation, propagation, and irradiation of tumor cell hybrids:

The procedure outlined below is a variation of the one reported by Prado et al (32) for the PEG-mediated fusion of somatic cells in monolayers. Thus, a preferred choice for fusing agent is high quality PEG-1450 (purchased from ATCC) which has been pretested for cytotoxicity.

Single-cell suspensions of patient-derived tumor cells ($1x10^7$ cells/100 mm dish) are plated on tissue culture dishes in DMEM supplemented with 10% FBS, streptomycin (100 µg/ml) and gentamicin (10 µg/ml). The following day, $4x10^6$ FO-1 #12 cells are added to each dish of patient-derived cells for co-cultivation. After 4-5 hours of co-cultivation, cells are rinsed twice with serum-free DMEM prewarmed at 37° (D37°), and exposed for 5

min to 50 µM sodium dodecylsulfate (SDS) in D37°. SDS-containing medium is suctioned off and the monolayer is treated with 3 ml/dish of 50% PEG in D37° for fusion. The PEG solution is suctioned off and the monolayer rinsed three times with D37° before adding complete medium containing 15 µg/ml hypoxanthine, 0.2 µg/ml aminopterin, 5 µg/ml thymidine (HAT). The day following PEG-mediated cell fusion, selection for tumor cells hybrids is started in complete medium containing HAT and 600 µg/ml of the neomycin analog geneticin (G418). Cell hybrids derived from the fusion of FO-1 #12 cells with patient-derived cells are selected by virtue of their HAT-resistant and neomycin-resistant phenotype and are propagated in selective medium for several weeks.

When fusing FO-1 #12 cells with cells in suspension [e.g., patient-derived peripheral blood lymphocytes (PBL)], a modification of the stirring protocol is used (33). Patient-derived cells (PML) and FO-1 #12 cells are washed by centrifugation in D37° and then mixed at an approximate 5:1 ratio (25 million PML:5 million FO-1 #12 cells). The resulting cell mixture is then spun at 300xg for 5 min in D37° containing 50 µM SDS. The mixed cell pellet is resuspended in 1 ml 50% PEG added slowly over 1 minute, and then stirred for an additional minute. Next, 10 mls D37° is slowly added over 2 minutes while stirring. The cell suspension is then centrifuged at 300xg for 5 min. The cell pellet is resuspended in complete medium containing 15 µg/ml hypoxanthine, 0.2 µg/ml aminopterin, 5 µg/ml thymidine (HAT). The day following PEG-mediated cell fusion, selection for cell hybrids is started in complete medium containing HAT and 600 µg/ml of the neomycin analog geneticin (G418). Cell hybrids derived from the fusion of FO-1 #12 cells with patient-derived PML

are selected by virtue of their HAT-resistant and neomycin-resistant phenotype and are propagated in selective medium for several weeks.

Cell hybrids derived from the fusion of FO 1-12 cells with patient-derived melanoma cells are selected by virtue of their HAT-resistant and neomycin-resistant phenotype and propagated in selective medium for several weeks. The HAT-resistant and neomycin-resistant cell population is then subjected to immunofluorescent staining using anti-HLA class I antigen mAb W6-32, followed by affinity-isolated fluorescein-labeled goat anti-mouse immunoglobulin. Mab W6-32 (corresponding hybridoma obtained through ATCC) is available as sterile ascites obtained from virus-free, immunodeficient (nude) mice and is used as a 1:1000 dilution in staining solution (full reactivity of W6-32 sterile ascites at 1:4000 dilution was documented). The surface expression by HAT-resistant and neomycin-resistant cells of HLA class I antigens confirms the presence of true hybrids. As an additional confirmation, tissue typing of patient-derived white blood cells and tumor cell hybrids is performed.

Determination of sterility and endotoxin activity:

Sterility, mycoplasma and endotoxin testing are initiated on the fin cell hybrid preparation for injection and on the autologous tumor cells and peripheral blood leukocytes used for skin tests. A gram stain is performed on the hybrid cells prior to injection. Mycoplasma testing can be performed utilizing the PCR-based detection kit manufactured by Stratagene (catalog #302007), which allows the identification of any of five strains of mycoplasma commonly associated with cell culture infections. Endotoxin testing can be performed using the

Limulus Amebocyte Lysate-based kit (Pyrogent Plus Gel-Clot LAL) manufactured by Bio-Whittaker (Walkersville, MD).

Preparation of irradiated hybrids for vaccination:

Approximately $5x10^6$ cell hybrids (sufficient for 1 injection of vaccine at the minimum dose) are washed three times in HBSS, resuspended in 4 ml of HBSS, tested for viability by trypan blue exclusion (at least 70% viability is preferred), and exposed to a single dose of 25 Gy γ-rays, sufficient to kill all cell hybrids. Irradiated cell hybrids are spun down at ≈ 250xg for 5 min and resuspended in 0.1 ml physiological saline before injection (I.D.). To insure uncompromised vaccine efficacy, the time-lapse between irradiation and vaccine administration should not exceed about two hours. Neomycin-resistant, HLA class I antigen-expressing hybrids are expanded and frozen down in aliquots of $6x10^6$ or more cells.

Samples of cell hybrids from each patient can be identified by some accepted identifier (e.g., the patient's initials followed by their hospital registration number and the letters FO-1).

Derivation of tumor-infiltrating lymphocytes (TIL) and peripheral blood lymphocytes (PBL):

TIL cultures are established as described by Yannelli et al. (34). Initial single-cell suspensions, containing tumor cells, lymphocytes, macrophages, and stromal cells ($5x10^5$ cells/ml), are seeded in 24-well culture plates (2 ml/well) in RPMI (Gibco-BRL) supplemented with 10% human AB serum, streptomycin (100 µg/ml), gentamicin (10 µg/ml), 2 mM L-glutamine, and interleukin-2 (IL-2, Cetus-Chiron, 6000 IU/ml). After 5-7 days, when the cell densities

exceed $1.5x10^6$ cells/ml, cultures are expanded and transferred to tissue culture flasks at a concentration of $5x10^5$ cells/ml in fresh medium. After 2-3 weeks, TIL cultures are tested for the surface expression of T cell markers (MHC class II, CD3, CD4, CD8) by flow cytometry using commercially available reagents (Coulter). TIL are cryopreserved in aliquots of $2x10^7$ cells/vial and stored in liquid nitrogen until use.

To obtain peripheral blood-derived lymphocytes (PBL), mononuclear cells are obtained from heparinized blood as described above and grown in AIM-V (Gibco-BRL) supplemented with 10% human AB serum, streptomycin (100 µg/ml), gentamicin (10 µg/ml), 2 mM L-glutamine, and interleukin-2 (IL-2, Cetus-Chiron, 6000 IU/ml) for 1-2 weeks. The cells can then be tested for the expression of T cell markers (MHC class II, CD3, CD4, CD8) by flow cytometry using commercially available reagents (Coulter) before using the cells in experiments of "education" with semi-allogeneic cell hybrids.

Education of patient-derived TIL or PBL and cytotoxicity studies:

For experiments of "education" of patient-derived TIL or PBL, semi-allogeneic cell hybrids derived from the same patient ($1x10^6$ cells in 3 ml of complete medium) are irradiated (25 Gy) and plated onto a 100 mm culture dish. After the cells are attached, medium is suctioned off and replaced with $5x10^6$ TIL or PBL in 10 ml of AIM-V medium without serum, with or without 120 IU/ml IL-2. As controls, identical cultures of TIL or PBL either exposed to irradiated parental FO-1 β_2 microglobulin$^-$ cells, or not exposed to any irradiated cells are established. After three to seven days, all three sets of TIL or PBL cultures

are tested for cytolytic activity against ^{51}Cr-labeled autologous target cells in ^{51}Cr release assays.

Depending on the experiment, target cells can be patient-derived tumor cell suspensions, patient-derived peripheral blood mononuclear cells (for example, in patients with HIV/AIDS or autoimmune disease), Epstein-Barr virus (EBV)-transformed B lymphocytes loaded with appropriate antigenic peptides (35), or other HLA-matched antigen-presenting cells, such as T2 cells.

Target cells are radiolabeled with ^{51}Cr in complete RPMI medium over a one to two hour period or overnight, depending on cell type. The targets are then incubated with the educated lymphocytes or controls in complete RPMI medium for 4-16 hours at effector to target ratios of 5:1 and 40:1. ^{51}Cr release into the supernatants is measured by a gamma counter. The percent lysis (% lysis) from the cytolysis assays is calculated by the following formula:

$$\% \text{ Lysis} = \frac{Ex - S}{M - S} \times 100$$

where Ex = experimental release of ^{51}Cr (cpm/min), S = spontaneous release of chromium-51 (cpm/min) by target cells, and M is maximum release of ^{51}Cr (cpm/min) by target cells when lysed by 0.1 N hydrochloric acid. As negative controls for the target cells, ^{51}Cr-labeled Daudi cells were used as targets for lymphokine-activated killer (LAK) cell activity and ^{51}Cr-labeled K562 cells as targets for natural killer (NK) cell activity, in order to ascertain that any change in cytotoxicity after exposure of lymphocytes to irradiated hybrids was T cell-mediated rather than being the result of increased LAK or NK cell activity (LAK and

NK cell activities are not HLA-restricted). The results of cytotoxicity experiments are shown in Tables 1 and 2. Values representing percent lysis are corrected for the percent lysis by each effector of ^{51}Cr-labeled Daudi cells used as a non-specific target.

Table 1 shows percent of TIL-mediated lysis of autologous melanoma cells (target) from patient JP1. Values shown were corrected for the % lysis by each effector of Daudi cells used as a target for lymphokine-activated killer (LAK) cells. [a]ND: not determined (JP1-TIL were growing poorly in the absence of stimulation with irradiated hybrids).

Table 1

	EFFECTOR:TARGET RATIO	
EFFECTORS	5:1	40:1
JP1-TIL (control + IL-2)	0.0	ND[a]
JP1-TIL (JP1 x FO1-educated)	11.0	67.0
JP1-TIL (JP1 x FO1-ed. + IL-2)	36.5	75.0
	% LYSIS OF AUTOLOGOUS TUMOR CELLS	

Table 2 shows the percent of TIL-mediated lysis of autologous melanoma cells (target) from patient GT1. In this experiment, a control was performed with GT1-TIL exposed to irradiated FO-1 parental cells transfected with the β_2 microglobulin gene. These FO1-β cells express allogeneic MHC class I molecules on the cell surface. This

experiment demonstrates that allogeneic stimulation per se does not enhance specific cytolytic activity by the TIL as does the semi-allogeneic stimulation by the GT1xFO1 hybrids. Values shown were corrected for the % lysis by each effector of Daudi cells used as a target for LAK cells.

Table 2

	EFFECTOR:TARGET RATIO	
EFFECTORS	5:1	40:1
GT1-TIL (control + IL-2)	5.0	19.2
GT1-TIL (FO1β-ed. + IL-2)	12.9	21.2
GT1-TIL (GT1 x FO1-ed. + IL-2)	16.0	42.7
	% LYSIS OF AUTOLOGOUS TUMOR CELLS	

Throughout this application various publications are referenced by numbers within parentheses. Full citations for these publications are as follows. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

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What is claimed is:

1. A cell having the characteristics of the cell line designated FO-1 #12, deposited with the American Type Culture Collection under accession number ATCC CRL-12177.

2. A cell deposited with the American Type Culture Collection under accession number ATCC CRL-12177.

3. The cell of claim 1, wherein the cell expresses a heterologous antigen.

4. The cell of claim 3, wherein the antigen is a tumor antigen.

5. The cell of claim 4, wherein the antigen is a melanoma antigen.

6. The cell of claim 5, wherein the melanoma antigen is gp100.

7. The cell of claim 4, wherein the tumor antigen is carcino-embryonic antigen.

8. The cell of claim 4, wherein the tumor antigen is a prostate cancer-specific antigen.

9. The cell of claim 8, wherein the prostate cancer-specific antigen is prostate-specific antigen.

10. The cell of claim 3, wherein the cell expresses an HIV-specific antigen.

11. The cell of claim 10, wherein the HIV-specific antigen is gag or an antigenic fragment thereof.

12. A fused cell hybrid of the cell of claim 3 and a mammalian cell.

13. The cell hybrid of claim 12, wherein the hybrid is irradiated.

14. The cell hybrid of claim 12, wherein the mammalian cell is a patient-derived human cell.

15. The cell hybrid of claim 14, wherein the patient-derived human cell is a white blood cell.

16. The cell hybrid of claim 14, wherein the patient-derived human cell is a patient-derived tumor cell.

17. A fused cell hybrid of the cell of claim 11 and a patient-derived human cell.

18. The cell hybrid of claim 17, wherein the patient-derived human cell is a white blood cell.

19. A method of treating a solid tumor in a patient, comprising administering to the patient the cell hybrid of claim 15, wherein the patient-derived white blood cell is derived from the patient being treated.

20. A method of treating a solid tumor in a patient, comprising administering to the patient the cell hybrid of claim 16, wherein the patient-derived tumor cell is derived from the patient being treated.

21. The method of claims 19 or 20, wherein the cell hybrid is administered in conjunction with a cytokine which can enhance the patient's immune response against the tumor.

22. The method of claim 21, wherein the cytokine is IL-2.

23. The method of claim 21, wherein the cytokine is granulocyte-macrophage colony-stimulating factor.

24. The method of claim 21, wherein the cytokine is IL-12.

25. A method of treating AIDS in a patient, comprising administering to the patient the cell hybrid of claim 18, wherein the patient-derived white blood cell is derived from the patient being treated.

26. A fused cell hybrid of the cell of claim 1 and a mammalian cell.

27. The cell hybrid of claim 26, wherein the hybrid is irradiated.

28. The cell hybrid of claim 26, wherein the mammalian cell is a patient-derived human cell.

29. The cell hybrid of claim 28, wherein the patient-derived human cell is a white blood cell.

30. The cell hybrid of claim 28, wherein the mammalian cell is a patient-derived tumor cell.

31. The cell hybrid of claim 30, wherein the patient-derived tumor cell is a melanoma cell.

32. The cell hybrid of claim 30, wherein the patient-derived tumor cell is a prostatic carcinoma cell.

33. The cell hybrid of claim 30, wherein the patient-derived tumor cell is a colon carcinoma cell.

34. The cell hybrid of claim 30, wherein the patient-derived tumor cell is a lung carcinoma cell.

35. The cell hybrid of claim 30, wherein the patient-derived tumor cell is a breast carcinoma cell.

36. The cell hybrid of claim 30, wherein the patient-derived tumor cell is a pancreatic carcinoma cell.

37. A method of treating AIDS in a patient comprising administering to the patient the cell hybrid of claim 29, wherein the patient-derived human cell is a white blood cell derived from the patient being treated.

38. A method of treating a solid tumor in a patient, comprising administering to the patient the cell hybrid of claim 30, wherein the patient-derived tumor cell is derived from the patient being treated.

39. The method of claim 38, wherein the cell hybrid is administered in conjunction with a cytokine which can enhance the patient's immune response against the tumor.

40. The method of claim 39, wherein the cytokine is IL-2.

41. The method of claim 39, wherein the cytokine is granulocyte-macrophage colony-stimulating factor.

42. The method of claim 39, wherein the cytokine is IL-12.

43. A method of making a cell hybrid, comprising the steps of:

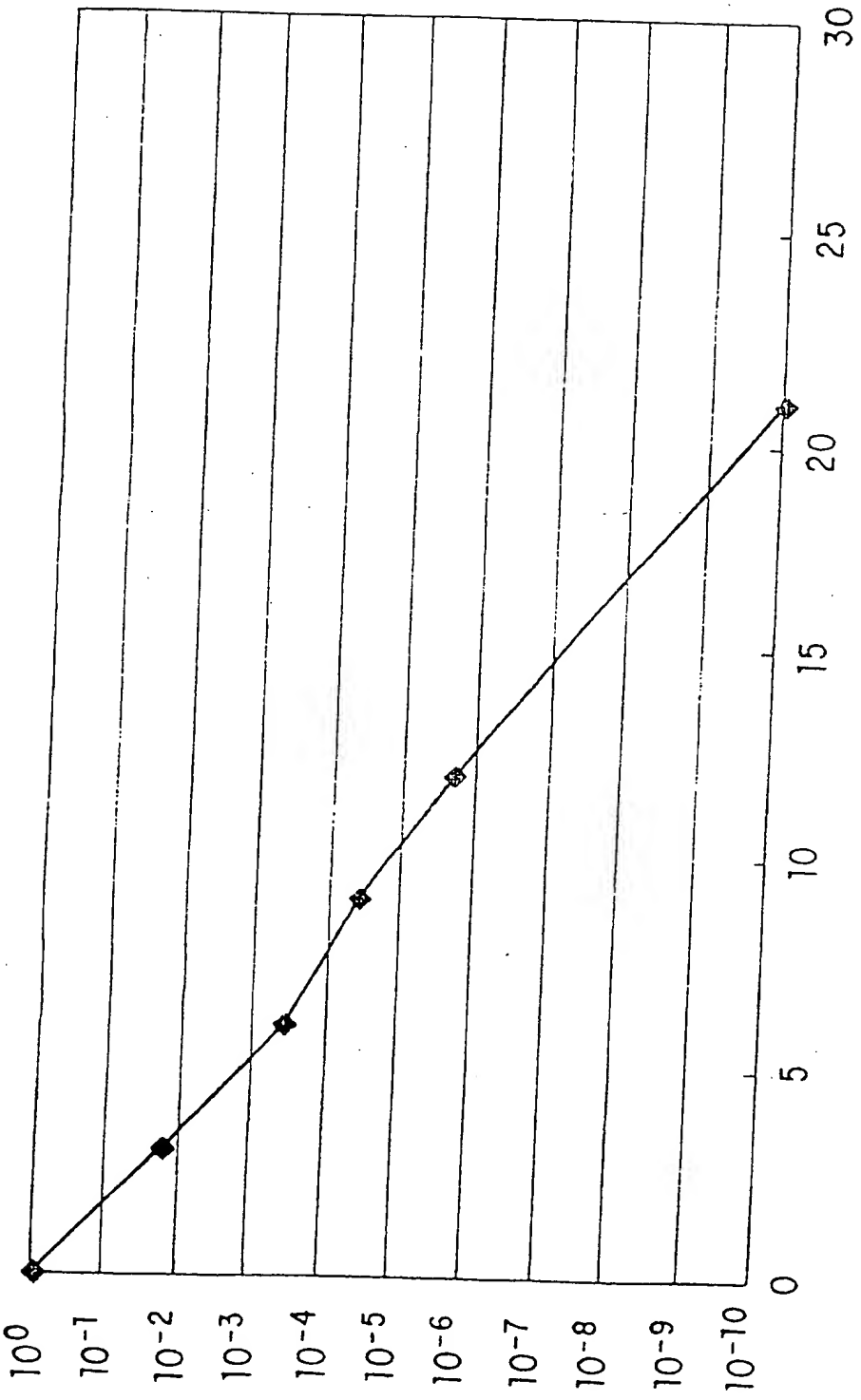
a) contacting a patient-derived cell with a cell deficient in β_2 microglobulin, having a selectable dominant marker and having a selectable recessive marker, under conditions in which the cells fuse to form a cell hybrid; and

b) screening for cell hybrids by determining the presence of the dominant marker and the presence of the recessive marker, whereby the presence of both the dominant and recessive markers is correlated with the presence of a cell hybrid.

44. The method of claim 43, further comprising the step of identifying cells that express HLA class I surface antigens, whereby the presence of HLA class I surface antigens is correlated with the presence of a cell hybrid.

45. A method of enhancing the proliferation and activation of a patient's cytotoxic T lymphocytes specific for tumor-associated, HIV/AIDS-associated or autoimmune disease-associated antigen targets, comprising contacting a population of lymphocytes from the patient with the cell hybrid of claims 13 or 27 .

46. A composition comprising a population of cytotoxic T lymphocytes produced by the method of claim 45.


SURVIVING FRACTION
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10^{-2}
10^{-3}
10^{-4}
10^{-5}
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10^{-9}
10^{-10}
0
5
10
15
20
25
30
DOSE (Gy)

FIG. 1

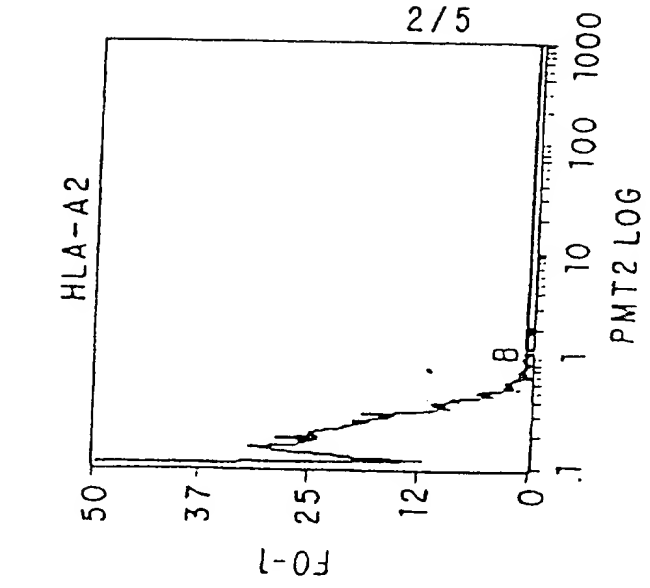

FIG. 2C

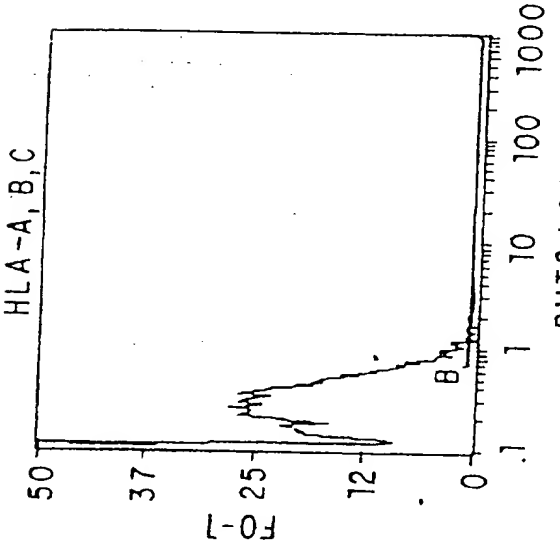

FIG. 2B

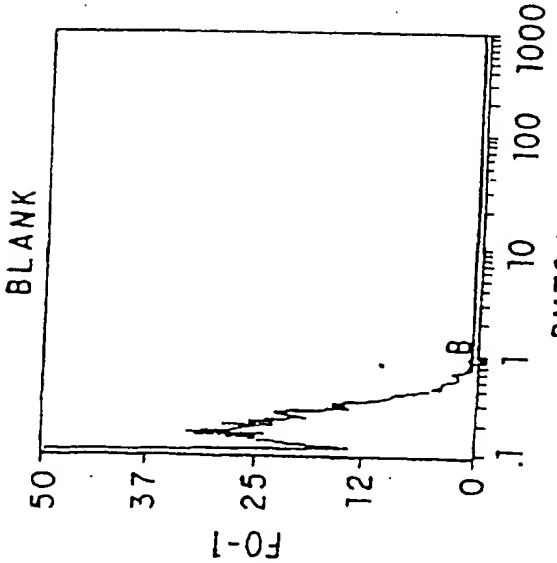

FIG. 2A

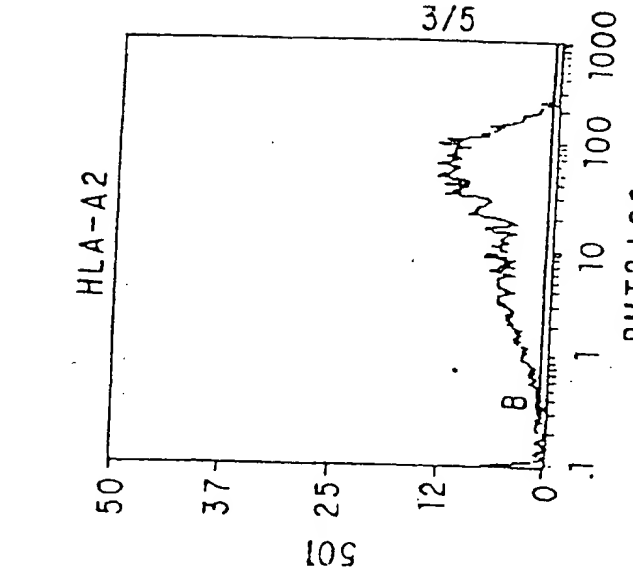

FIG. 2F

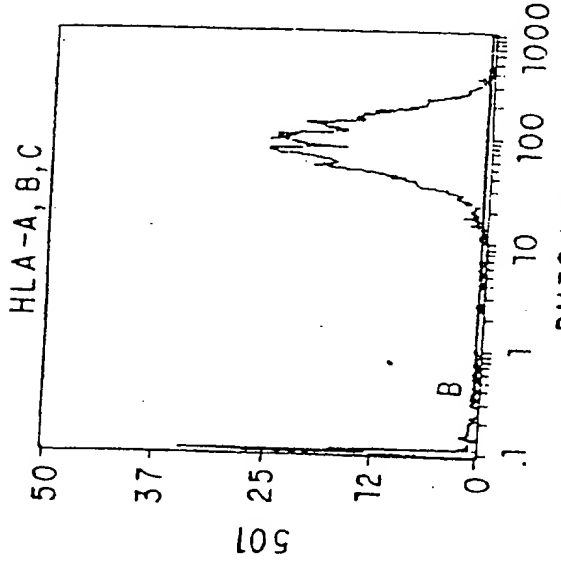

FIG. 2E

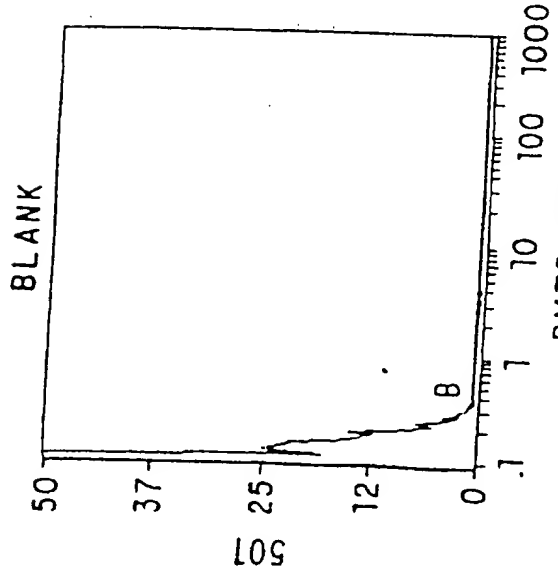

FIG. 2D

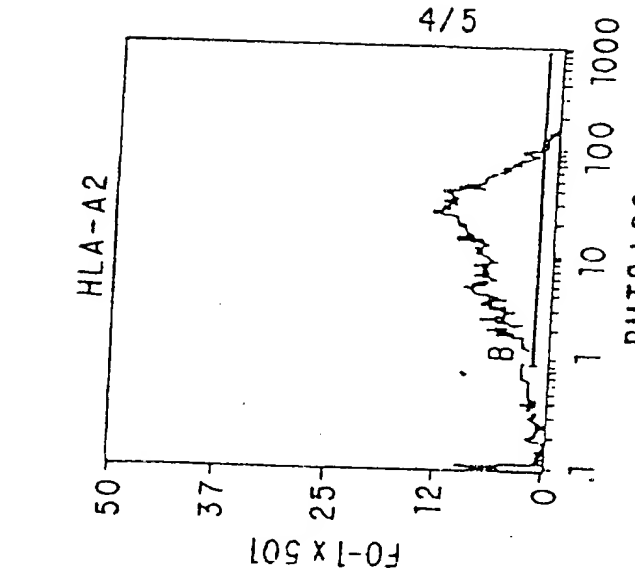

FIG. 2I

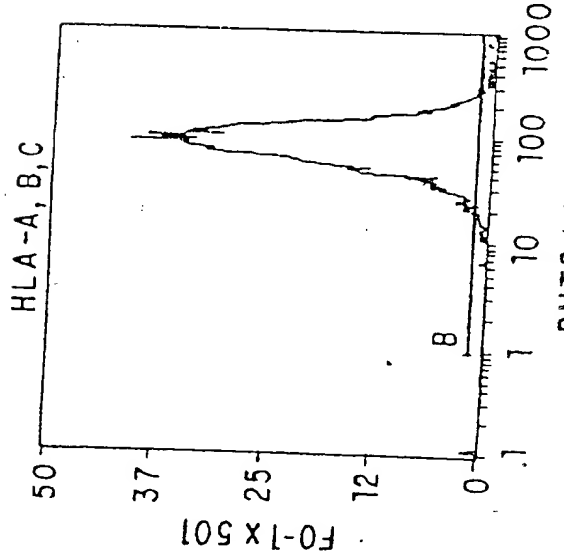

FIG. 2H

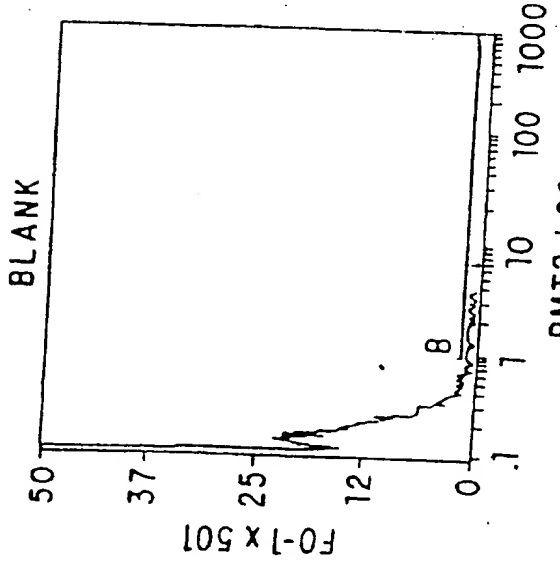

FIG. 2G

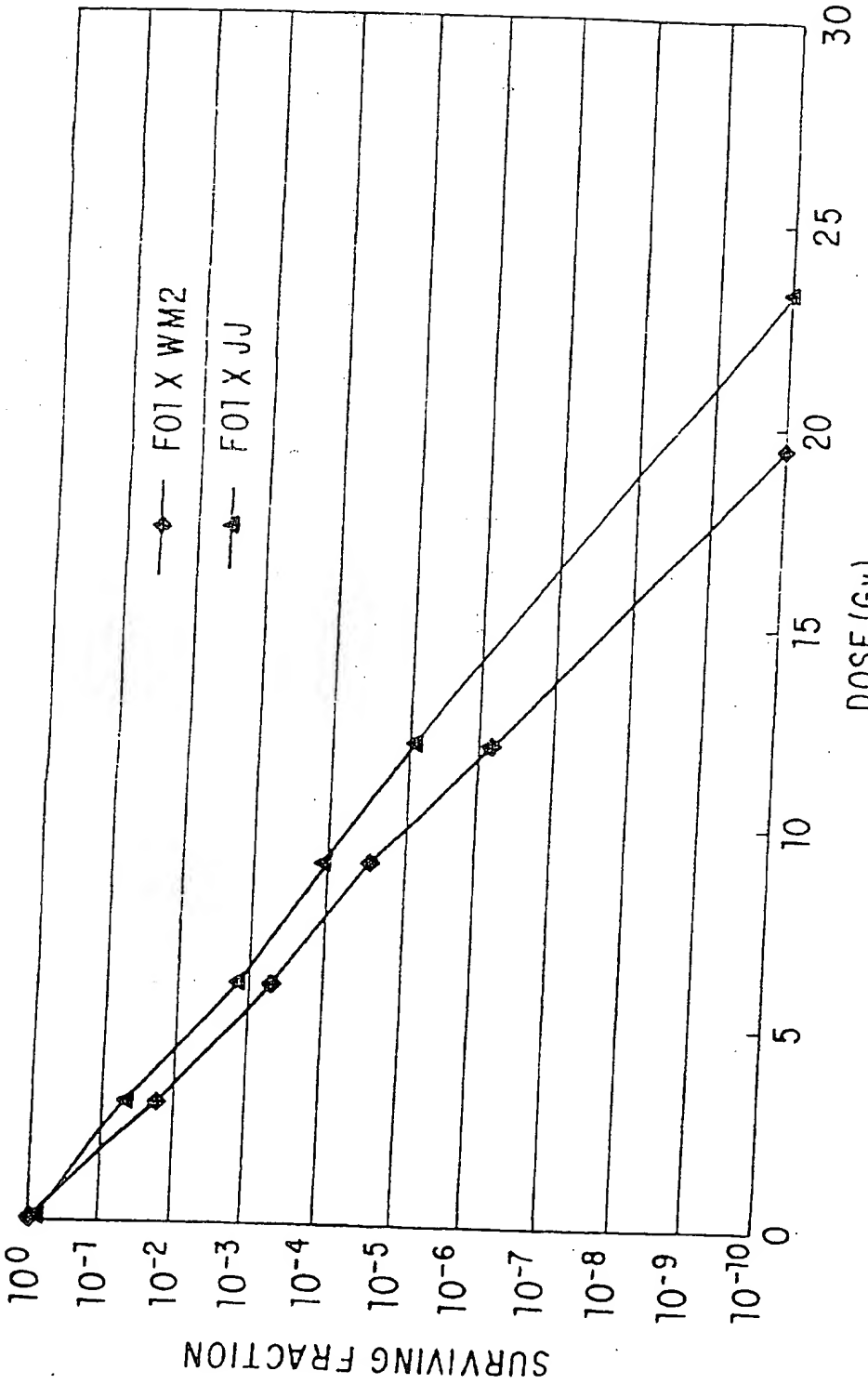
F01 X WM2
F01 X JJ
SURVIVING FRACTION
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
10^{-8}
10^{-9}
10^{-10}
0
5
10
15
20
25
30
DOSE (Gy)

FIG. 3

INTERNATIONAL SEARCH REPORT

Intern ial Application No
PCT/US 97/15920

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N5/06 C12N5/22 C12N15/07 //C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
Y	C.M. D'URSO ET AL.: "Lack of HLA class I antigen expression by cultured melanoma cells FO-1 due to a defect in B2m gene expression" THE JOURNAL OF CLINICAL INVESTIGATION, vol. 87, January 1991, NEW YORK US, pages 284-292, XP002051964 cited in the application see the whole document	1
A	--- -/--	2,3,12, 17,26

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 January 1998	11. 02. 98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	De Kok, A

INTERNATIONAL SEARCH REPORT

Intern ial Application No
PCT/US 97/15920

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
Y	K. HOSOI ET AL.: "A human-mouse hybrid cell line expressing both human leucocyte and histocompatibility-2 antigens" JAPANESE JOURNAL OF PHYSIOLOGY, vol. 40, no. 2, 1990, NAGOYA JP, pages 297-304, XP002051965	1
A	see the whole document	12, 14-16, 26,43
A	WO 93 07887 A (CELL GENESYS INC) 29 April 1993 see page 1 - page 3 see page 4, line 10 - page 5, line 28 see page 8, line 27 - page 9, line 10 see page 14, line 9 - line 21	1-11
A	WO 95 16775 A (Y. GUO ET AL.) 22 June 1995 see page 2, line 31 - page 13, line 21 see claims 1-20,29-51	1-9, 12-20, 26-38
A	D.L. TOFFALETTI ET AL.: "Augmentation of syngeneic tumor-specific immunity by semiallogeneic cell hybrids" JOURNAL OF IMMUNOLOGY., vol. 130, no. 6, June 1983, BALTIMORE US, pages 2982-2986, XP002051966 cited in the application see the whole document	12-16, 19,20, 45,46
A	WO 95 31208 A (BOEHRINGER MANNHEIM GMBH) 23 November 1995 see page 3, last paragraph - page 5 see page 6, last paragraph - page 7	1,21,22, 39,40, 43,45,46
A	EP 0 197 489 A (BEHRINGWERKE AG) 15 October 1986 see abstract	1-3,43
P,X	D.A. NEWTON ET AL.: "Melanoma cell hybrids as cancer vaccines" PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, vol. 38, March 1997, WASHINGTON US, page 398 XP002051967 see abstract no.: 2671	1-46

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/15920

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons

1. [X] Claims Nos.
because they relate to subject matter not required to be searched by this Authority, namely

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically

3. [ ] Claims Nos.
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.